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CODLING MOTH:
AGAR SUBSTITUTES IN ARTIFICIAL DIETS

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ABSTRACT

The following materials and combinations thereof were tested as substitutes for agar in artificial diets used to rear the codling moth, *Laspeyresia pomonella* (L.): Gelatin; algins; carrageenan; Furcellaran; guar and locust bean gum; carboxymethylcellulose (CMC); Gelgard; pectin L.M.; corn, potato, and wheat starch; glutin; casein; and soya lecithin. Diets were gelled by each product listed except casein and CMC, but moisture retention often was inadequate. However, desiccation and syneresis were controlled by combining one or more of these products. Starches, stabilized with small amounts of other colloids, were the most promising of the gels tested, particularly in an economic sense.

KEYWORDS: *Laspeyresia pomonella* (L.), codling moth, artificial diets, gelling agents, agar substitute, gelatin, algin, carrageenan, Furcellaran, guar gum, locust bean gum, carboxymethylcellulose (CMC), Gelgard, pectin L. M., corn, potato, wheat starch, glutin, casein, lecithin.

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CODLING MOTH: AGAR SUBSTITUTES IN ARTIFICIAL DIETS

By J F. Howell[1]

INTRODUCTION

Artificial diets for rearing lepidopterous larvae are characteristically solid (as compared to liquid) with a high moisture content (*9*).[2] Moisture not absorbed by the particulate materials present is bound colloidally. Codling moth, *Laspeyresia pomonella* (L.), larvae naturally burrow in the medium, which must be firm enough to prevent collapse of the burrows. Syneresis (water seeping out of the diet) must be completely controlled to prevent the burrows from filling with fluid (firmness of gels and syneresis are not always directly related--a firm gel can exhibit syneresis). The required firmness and freedom from syneresis are typically obtained by using agar.

The demand created for large numbers of inexpensive insects by modern control programs, such as the sterile male technique and parasite production, has necessitated examination of the cost of artificial diets. Wherever possible, ingredients are eliminated and substitutes are sought for the more expensive essential items. In the earlier codling moth diets (*2*), 47 percent of the cost was for agar; therefore, at the Yakima Agricultural Research Laboratory, numerous materials have been tested as substitutes. Although not all of these materials are useful for codling moth rearing, they are described because they may be used to culture other insects.

METHODS AND PROCEDURES

Basically, the diets were prepared as outlined by Howell (*3*). Gelatin was dissolved by heating it in water. Hardening agents (ketones) were added and mixed with the gelatin before dissolution. All phycocolloids were dissolved by heating in suspension in water. Starches or gums combined with the phycocolloids were either pregelatinized (cold water soluble) or dissolved by heating in water before being incorporated with the phycocolloids. Guar gum was dry mixed with borax, and the mixture was sifted into and mixed simultaneously with the fully prepared, heated diet. Another variation was to prewet the guar with alcohol to prevent lumping before adding it to the diet. Using this procedure, borax had to be added separately. Locust bean gum was dissolved in hot water and used just as agar. Borax was mixed into the diet following the adding and mixing of the gum. Carboxymethylcellulose (CMC) was prewetted with alcohol to prevent lumping, stirred into cold water, and the solution was heated until completely fluid at which time it was mixed with the other ingredients. Alternately, the wetted solution was added to the diet and the whole diet heated to 65° C. Gelgard was wetted with alcohol and mixed with the other ingredients by using a slow-speed food mixer (do not use a blender). Pectins were dissolved by heating in solution. Starches were used in two ways: (1) By heating alone in water to hydrate and then adding to the other ingredients, and (2) by suspending in cold water, mixing into the diet, and heating the entire mixture. Finally, glutin, casein, and soya were tested in diets, and the complete mixtures were heated.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 8.

The diets were prepared in 3.8-liter batches, poured into steel trays lined with plastic liners (*3*), and surface coated with paraffin (*1*). Standard agar preparations (*3*) were used as controls for comparison with other preparations. Eggs were prepared and placed on the diet. Texture and condition of the diet at 25 days and number of moths produced were the criteria used to judge each diet. There were two replications for each preparation.

RESULTS AND DISCUSSION

Gelatin

The freezing point (the temperature at which a gelatin sol converts to a gel) of table-grade gelatin (200 to 225 bloom grams) is about 25° to 35° C depending on concentrations and grade. At the low concentration tested (table 1), table-grade gelatin has been a useful gelling agent in insect diets only if hardened with a ketone, which raises the freezing point. Hardening with formaldehyde (20 ml/77 g gelatin) was not always successful, but gelatins with higher gel strengths (275 bloom grams or higher) and freezing points above 32° C are available and do not require hardening. Diets made with gelatin or with gelatin plus wheat starch (table 1) had adequate gel strength, but they desiccated excessively. Gelatin diets would be applicable for insects with short maturation periods or in applications where the diet is renewed or changed regularly (external feeders). Tests to control desiccation by making appropriate mixtures of gelatin and agar (or other colloids) in addition to using humectants, such as propylene glycol or inverted sugars, have not been made. The high protein content of gelatin plus economic benefits (about one-half the cost of agar) make this material a very useful gelling agent.

TABLE 1.--*Evaluation of various gelling agents used in 3.8-liter batches of codling moth diet*

Thickening agents	Percentage of dry ingredient	Average number of moths produced per tray	Condition of diet at 25 days and changes in diet contents as noted
Gelatin	2.00	0	Desiccated rapidly.
Do.	1.90	46	Desiccated; leathery dry.
Gelatin + wheat starch	1.00 5.00	0	Failed to gel.

TABLE 1.--*Evaluation of various gelling agents used in 3.8-liter batches of codling moth diet*--Continued

Thickening agents	Percentage of dry ingredient	Average number of moths produced per tray	Condition of diet at 25 days and changes in diet contents as noted
Gelatin +	1.00	90	Desiccated.
wheat starch	10.00		
Do.	1.00	131	Desiccated but not as rapidly as gelatin alone.
	9.40		
Agar	1.25	146	Good texture.
Gelcarin[1]	1.60	131	Do.
Gelcarin +	.50	106	Good texture; some cracking.
wheat starch	5.00		
Furcellaran[1]	2.00	0	Uncontrolled syneresis.
Furcellaran +	.20	97	Desiccation and some syneresis.
wheat starch	7.50		
Do.	1.00	192	Good texture.
	7.50		
Guar	.90	0	Poor gel.
Guar +	.90	0	0.02 percent borax toxic.
borax	.02		
Guar +	.40	160	Good texture.
agar	1.10		
Guar +	.90	247	Do.
agar +	.30		
potato starch	1.25		
Locust bean gum +	.40	172	Gel softened with aging.
agar	1.20		
Locust bean gum +	.50	0	0.05 percent borax toxic; diet softened with aging.
potato starch +	2.10		
borax	.05		
CMC +	1.00	0	Failed to gel.
aluminum acetate	.15		
CMC +	.20	0	Soured.
casein	10.00		
CMC +	.50	13	Desiccated.
wheat starch	10.00		
Gelgard[1]	2.50	22	Desiccated rapidly.
Gelgard +	2.50	9	Desiccated; tough.
CMC +	1.00		
casein 11-A	3.10		
Gelgard +	.90	123	Some cracking; fair texture.
wheat starch	10.00		
Do.	1.00	91	Some cracking resulting in desiccation.
	12.50		

TABLE 1.--*Evaluation of various gelling agents used in 3.8-liter batches of codling moth diet*--Continued

Thickening agents	Percentage of dry ingredient	Average number of moths produced per tray	Condition of diet at 25 days and changes in diet contents as noted
Gelgard + wheat starch	1.00 7.50	264	Some cracking but with drying. Wheat germ content of diet increased to 8.7 percent.
Pectin L.M.	1.00	0	Uncontrolled syneresis.
Pectin L.M. + wheat starch	.20 10.00	87	([2]).
Pectin L.M. + CMC + wheat starch	.50 .10 7.50	87	Poor texture; too hard and tough.
Wheat starch	7.50	0	Cracked excessively and desiccated.
Wheat starch + agar	2.50 1.20	79	Some cracking and desiccation.
Wheat starch + CMC	7.50 .70	239	Good texture. Wheat germ increased to 11.2 percent.
Do.	8.70 .70	85	Do.
Do.	13.30 .10	50	Cracking and desiccation.
Do.	12.50 .25	277	Good texture. Wheat germ increased to 11.2 percent.
Wheat starch + CMC + agar	5.00 .10 .50	0	Syneresis developed as this formulation aged.
Glutin + wheat starch	2.50 10.00	89	Cracked excessively.
Casein 11-A + CMC + wheat starch	3.10 .50 7.50	4	Uncontrolled syneresis.
Soya + wheat starch	15.00 5.00	119	Some cracking. Casein was omitted from the diet.

[1] Registered trademark.
[2] Condition not noted.

Seaweed Extracts

Phycocolloids tested in this category were agar, algin, carrageenan, and Furcellaran. Agar gel strength commonly ranges from 500 to 600 but an 800-gel strength is available. Refinement usually relates to clarity and freedom from impurities, not gel strength. Agars are blended to provide consistent gel strengths, and the gel strength of the product should be considered when purchasing. Agar prices are extremely unstable (see Appendix).

Sodium, potassium, ammonium, and propylene glycol ester alginates are soluble in cold or hot water, but calcium alginate has limited water solubility. Alginates can be degraded by overheating, resulting in a loss of viscosity. Gels are formed in cold water if calcium or other polyvalent metal cations are present in sufficient concentration. The chemical gels thus formed are not reversible or elastic. These gels are somewhat pH dependent (*10*). Sodium alginate was used successfully at Yakima for codling moth and has been used extensively by Moore and Navon (*7*, *8*). Their results illustrate the utility of this material, and for brevity my results are omitted. It is more moderately priced than agar (see Appendix).

Carrageenan (Gelcarin HWG) is partially soluble in cold water but must be heated for complete solubility. Carrageenan forms elastic gels in the presence of metallic cations such as potassium, calcium, and ammonia. Locust bean gum improves the elasticity of these gels. Carrageenan has a high degree of reactivity with certain proteins (milk protein), and strong gels are formed with extremely low concentrations of this colloid. Codling moth diets with high casein content and carrageenan fail to gel, but carrageenan alone may be used to replace agar with considerable savings. Moisture retention and texture are comparable to agar preparation, as is yield of moths. Carrageenan has been used for some time in mass rearing codling moths at Yakima. Some difficulty in mixing is experienced because of the partial solubility of this material in cold water. Incidence of wing deformities associated with this material is low.

Furcellaran is soluble in hot water and forms an elastic gel similar to agar. Gel strength is pH dependent. Acidic conditions (pH below 5.0) produce soft gels and loss of gelling power. The desirable pH range is from 6 to 7. Furcellaran gelled codling moth diet, at pH 5, was plagued by uncontrolled syneresis. Guar gum and starch were used to control syneresis. Here, when combined with wheat starch, the mixture provided a satisfactory gel. A low incidence of wing deformities occurs when Furcellaran is used. Furcellaran is half the price of agar (see Appendix).

Guar and Locust Bean Gums

Guar gum hydrates rapidly in cold water and forms very viscous solutions. Locust bean gum partially hydrates in cold water and requires heating for maximum solubility. Both gums form firm gels with the addition of borax; however, in the amounts used, borax was toxic to codling moth larvae. Each of these gums stabilize other materials (seaweed extracts and starches) to control syneresis. These gums are inexpensive (see Appendix), produce firm gels at low concentrations, and are potentially useful as a substitute gelling agent. Guar is used most effectively in combination with gelling agents, particularly starch.

Carboxymethylcellulose (CMC)

CMC dissolves in cold water and forms a viscous solution. Gels are produced with the addition of trivalent cations such as aluminum acetate. CMC is incompatible with ingredients in the codling moth diet, being degraded (softens with age) by ascorbic acid, Wesson mineral salt mixture, casein, and wheat germ. CMC has routinely been used to thicken the codling moth diet to prevent separation and settling out of the particulates until the agar cools and gels (5).

Gelgard

Gelgard is a cross-linked acrylamide acrylic polymer. The polymer is not water soluble but absorbs 600 to 800 times its own weight of solution and transforms into a viscous jellylike product. High shear mixing destroys some of the thickening properties of this material by breaking the polymers. Concentrations of 2 to 3 percent thicken the codling moth diet, but the diet dries out too rapidly. Gelgard has been used successfully with other gums.

Low Methoxyl Pectin

Regular or high methoxyl pectins are dependent on sugar, high solids content, and a specific pH (near 3.5) for gel formation. In contrast, low methoxyl pectin (pectin L.M.) gels in the presence of certain polyvalent ions (calcium over a pH range from 2.5 to 6.5 with best gels in the range of 3.2 to 4.0). Moreover, this gelling is independent of sugar and solids content. Pectin L.M. was used successfully in diets for rearing codling moths, but syneresis was a problem. Syneresis was controlled by the addition of either Gelgard, starch, or CMC.

Starch

Starches hydrated in hot water form viscous solutions that gel when cooled. Overheating or subjecting cornstarch to the shearing action of high-speed blenders destroys the gelling properties of this starch. However, this is not the case with wheat starch. Starches are modified in many ways to alter hot and cold viscosities and gelling. Natural cornstarch substituted for agar does not support normal growth and development of the codling moth larvae. However, acid-modified potato starch and natural wheat starch have been used successfully. Wheat starch is especially useful since it cannot be overheated easily, and the granules do not rupture to any extent in a high shear blender. The problems associated with starch gels were cracking, desiccation, and some syneresis. Cracking, which accelerates dehydration, is controlled by stirring the diet after it has partially cooled (taking advantage of the thixotropic property of starch; that is, a tendency to thin when stirred yet abruptly thickens when stirring stops)(*4*). Those pregelatinized starches (cold water soluble) tested were not satisfactory gelling agents for codling moth diets because higher concentrations were required even for soft gels. However, pregelatinized products may be readily adapted to other diets. Combinations of pregelatinized starches and cold water soluble gums (guar, carageenan, and algin) were not tested.

Glutin, Casein, and Soya

Glutin has been used unsuccessfully as a binder to give elasticity and cohesiveness to the gels to reduce cracking and to prevent desiccation. Modified caseins that thicken water solutions by absorption alone or in combination with gums (table 1) were not suitable. High casein diets soured. Soya has a high lecithin content. This lecithin hydrates and swells in water, forming a collodial solution, which thickens the diet mixture. Soya in combination with starch has been used to rear codling moth (*4*).

SUMMARY

High gel strength gelatins (275 to 300 bloom), all phycocolloids tested, pectin L.M., Gelgard, and starch alone or in combination with other gums or colloids have been used with some success to replace agar in codling moth diets. Casein and glutin are not appropriate for codling moth diet but may be applicable when firm gels are not necessary. CMC, guar, and locust bean gum can be used alone, but in codling moth diets they must be used in combination with other gums and colloids.

When cold water soluble colloids are used, special attention must be given to the mixing procedure to insure that ingredients are thoroughly mixed before gelation proceeds to the point that good distribution is not possible (sodium alginate, guar, and some carrageenans). With cold water solubility, getting the colloid itself into solution requires special attention. Wetting agents (such as alcohol) need to be used, otherwise lumping occurs and much of the product is wasted. Lumping was especially prevalent with CMC, Gelgard, and guar gum.

Cracking with accompanying desiccation was particularly troublesome with starch. Cracking was a phenomenon associated with cooling; the top of the diet cools and contracts more rapidly than the bottom. Late in these tests, the thixotropic properties of starch were used to control cracking.

Cost comparisons are available in the Appendix. Starches are, without question, the choice material from an economic standpoint, followed by lecithin (in soya meal) and guar gum. Combinations of gelling agents that entomologists have not been inclined to test, such as starch, guar, and algin, are very promising from an operational and economic standpoint. Recent advances in starch chemistry (*6*, *11*, *12*) have perhaps provided a starch that will not require heating. However, most pregelatinized products have been heated in manufacture and will be reflected in the purchase price.

Starches are nutritionally active, whereas most phycocolloids are nutritionally inert. This characteristic will probably be the limiting factor for using starch as a gelling agent for lepidopterous insects. Starch and soya were successfully combined to produce an excellent codling moth diet (*4*). Starch cooks slowly in open nonpressurized cookers, but with pressurized jacketed cookers this is no problem.

At present, the most promising area for agar substitutes lies in combining various materials to produce the gel with characteristics fitting the species being reared. Starches, guar gum, algin, and lecithin deserve serious consideration. Particular attention should be given to moisture retention. My experience has been that the moisture level in the diet is as critical, perhaps even more critical, than the food ingredients. Moisture retention is closely linked with the colloidal properties of the gel agents used. Concentration and size of particulates in the diet are also extremely important relative to moisuture retention. Often it is difficult to separate dietary deficiencies from moisture deficiencies.

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APPENDIX

Cost[1] and Source of Gelling Agents

Material	*Cost per pound*	*Source*
Gelatin:		
275 bloom	$ 2.21	Swift and Company
300 bloom	2.33	Swift and Company
Agar	5.75 11.50	Burtonite Company U.S. Biochemical Company
Alginate:		
Sodium	1.79	Hathaway Allied Products
Calcium	2.12	Kelco Company
Carrageenan (Gelcarin HWG)	3.50	Marine Colloids
Furcellaran	3.25	Burtonite Company
Guar gum	.58	Burtonite Company
Locust bean gum	.95	Hathaway Allied Products
Carboxymethylcellulose	[2]1.14-2.00	Hercules, Inc.
Gelgard (discontinued product)	2.00	Dow Chemical Company
Pectin L.M.	3.11-3.25	Sunkist Growers, Inc.
Corn starch	.113	A. E. Staley Company
Potato starch	.150	A. E. Staley Company
Wheat starch	0.145-0.155	General Mills, Inc.
Glutin	.57	General Mills, Inc.
Casein	.72	American Casein Company
Soya lecithin (soya meal)	.146	Western Farmers Assoc.

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[1] All costs are current (spring 1976) prices.
[2] Depending on quantity.

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